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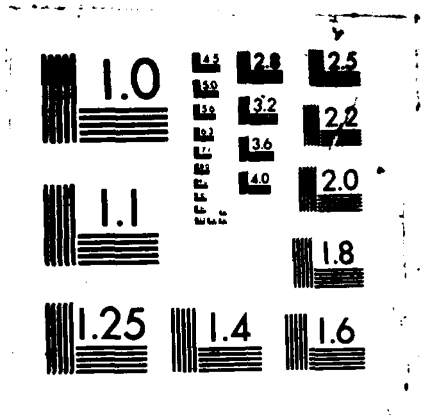

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BIOSYSTEMATICS OF AEDES (NEOMELANICONION)

For the period 5 May 1986 - 4 May 1987

Annual Report

Thomas J. Zavortink

June 1987

Supported by

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19. Abstract

The objective of the "Biosystematics of Aedes (Neomelaniconion)" project is to produce a modern taxonomic monograph of the aedine subgenus Neomelaniconion by using comparative morphological taxonomic procedures and characteristics from both sexes and all stages of the life cycle.

During the first contract year, facilities, specimens, equipment, and supplies were obtained, and staff was hired and trained. Specimens were acquired through loans from museums and through field work by staff and cooperators in Australia, Ivory Coast, Kenya, and Zambia. Nearly 150 clutches of eggs were subjected to standard hatching procedures in the laboratory in San Francisco and many progeny series were reared. Nearly 150 field collections were processed. Approximately 3500 adult mosquitoes, 2300 immature mosquitoes, and 70 male genitalia have been prepared for study, and most of these have been labeled. All specimens of Neomelaniconion have been tentatively identified and 21 species are represented. Preliminary drawings of one species of Neomelaniconion were nearly completed. Much of the necessary taxonomic literature was obtained and preliminary taxonomic work was begun. Conclusions related to field work are that collecting soil samples is not an efficient way to obtain Neomelaniconion and ~~that~~ rearing progeny in the laboratory from eggs laid by females blooded in the field yields the best and most useful specimens. Conclusions related to the taxonomic work are that much additional field work must be done in order to obtain research quality material; that Neomelaniconion is very difficult taxonomically, with sympatric species often are so variable that it may not be possible to prepare simple keys to distinguish females and larvae of them; ~~that~~ taxonomic problems exist with the specific limits of fuscinervis and of palpalis and the species related to it or similar to it; and that the eggs of Neomelaniconion may provide valuable taxonomic characters.

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Contents

Statement of the Problem

The ultimate objective of the project "Biosystematics of Aedes (Neomelaniconion)" is to produce a modern taxonomic monograph of the subgenus Neomelaniconion Newstead of the genus Aedes Meigen. This group of mosquitoes, which is primarily Ethiopian in distribution, is involved in the transmission of several arboviruses, the most important of which is Rift Valley fever virus. The subgenus Neomelaniconion has never been the subject of a thorough taxonomic study, and so its species remain poorly and inadequately known. Both the lack of basic information on the number of species in the group and the lack of reliable keys for the identification of these species severely hamper the acquisition and reporting of biological information about these mosquitoes. The result is that the distribution, bionomics, and disease vector potential of the different species remain unknown or uncertain. This in turn prevents a complete understanding of the natural history of Rift Valley fever virus, which may be maintained between epizootics by transovarial transmission in species of Neomelaniconion.

Background

As it is presently understood, the subgenus Neomelaniconion includes 28 nominal species, 24 of which are considered to be valid taxonomic species or subspecies (1-3). All except one of the currently recognized species are restricted to the Ethiopian Region. The exception is Aedes lineatopennis (Ludlow), which is widespread in the Oriental and Australian regions.

The existing taxonomy of the subgenus Neomelaniconion dates back to Edwards's treatment of the group (under its former name, Banksinella Theobald) in his catalog of the family Culicidae (4) and in his volume on adult Mosquitoes of the Ethiopian Region (5). Edwards's studies were based almost entirely upon adult mosquitoes, and characteristics of the immature stages were not considered. In the many decades since Edwards's brief taxonomic treatments of Neomelaniconion, there has been no comprehensive study of the group. Several additional species have been described (3, 6-10), immatures of a few species have been partially described or illustrated (7, 9, 11-17), one nominal species has been transferred to the subgenus (18), and two nominal species have been removed (19).

In the absence of a comprehensive study of Neomelaniconion, the subgenus remains poorly and inadequately known. The immature stages, in particular, have been

neglected. They have never been used to help define the species of the group or to help place these species into a natural classification. In fact, to this day the immatures of nearly half the species of Neomelaniconion are unknown, and for those species in which they are known, they have been described and illustrated very superficially. The complete larval and pupal chaetotaxy has not been studied for a single species. Available keys to adults (5, 9, 15) and larvae (11, 15) of Neomelaniconion are inadequate because they treat only a portion of the species now known or treat only the species of a restricted region.

Numerous arboviruses have been isolated from species of Neomelaniconion (20). The virus that causes Rift Valley fever, an important disease of domestic animals and humans in Africa and a potential international disease problem (21), is the most important of these. This virus has been isolated from two species of Neomelaniconion, circumluteolus (Theobald) and lineatopennis (probably mcintoshi Huang) (22, 23). Recent studies on Rift Valley fever in Kenya have provided evidence that lineatopennis (again, probably mcintoshi) is a reservoir for the virus between epizootics, transmitting it transovarially from generation to generation (24). This discovery underscores the importance of obtaining basic information on the systematics and biology of species of Neomelaniconion, for such information is critical to a complete understanding of the natural history of Rift Valley fever virus.

Approach to the Problem

A modern systematic study of Neomelaniconion, utilizing morphological characteristics from both sexes and all stages in the life cycle, will be undertaken in order to determine the number of species in the subgenus, the most reliable means of distinguishing these species from each other, the existence and nature of intraspecific variation, the geographic distribution of the species, and the evolutionary relationships of the species. The results of this study will be published in a monograph that will include: taxonomic descriptions of species and groups of species; identification keys for all stages in the life cycle; detailed drawings of the larva, pupa, and male genitalia of each species and of the adult morphology for selected species; photographs of eggs; information on type specimens; synonymies; discussions of diagnostic characters, variation, and relationships; summaries of bionomics and medical importance; data on geographical distribution of the species, including lists of specimens examined and maps; and a bibliography.

Although the historically important specimens of Neomelaniconion currently held in museums will be examined, the bulk of the specimens studied will be collected specifically for the project. The collection, rearing, and preservation of material and the recording of field data will follow the procedures developed for the "Mosquitos of Middle America" project (25). Emphasis will be placed on obtaining the immature stages of species of Neomelaniconion and on associating the sexes and stages of the species by means of individual or progeny rearings. Specimens collected in the field or borrowed from museums will be prepared for study using standard laboratory procedures for mosquitoes, in general following the methods of Belkin (19). Classical, comparative morphological taxonomic procedures will be used, as outlined for mosquito systematics by Belkin (19) and Zavortink (26). The form of presentation and terminology used in the final monograph will follow Belkin (19) and Zavortink (27-29) in large part.

The initial phases of research on the "Biosystematics of Aedes (Neomelaniconion)" project must, out of necessity, emphasize training of staff, development of field and laboratory techniques, field work to collect and rear specimens, and laboratory work to prepare the specimens for critical study.

Results and Discussion

All accomplishments related directly or indirectly to the ultimate goal of producing a monograph of the subgenus Neomelaniconion that were completed during the first contract year of the project "Biosystematics of Aedes (Neomelaniconion)" are described below.

FACILITIES

In addition to the Principal Investigator's laboratory of approximately 150 square feet of work space, two additional laboratories were acquired for the project. One of these, measuring 260 square feet, contains most of the equipment and supplies for the project and is the workplace of the taxonomic research specialist. The other laboratory, with 275 square feet total, includes an inner room of 45 square feet and is utilized for rearing mosquitoes.

All major pieces of equipment for which funds were provided have been acquired for the project and are in operation. All supplies, tools, and chemicals necessary for the first year's collection, rearing, and specimen preparation activities were obtained.

STAFF

The following full-time and part-time staff are supported by the contract:

Thomas J. Zavortink, Principal Investigator (50% time)
Sandra S. Shanks, Taxonomic Research Specialist (100% since 23 May 1986)
Mary Ann Tenorio, Scientific Illustrator (Piecework, since 15 April 1987)

Ms. Shanks has been fully trained in the procedures for collecting mosquitoes in the field, rearing mosquitoes, and in preparing mosquito specimens for scientific study. She does an outstanding job, and in a relatively brief time she has become the finest preparer of microscope slides of individually reared larval and pupal skins of mosquitoes working today. She is also fully familiar with the operation of the microcomputer acquired for the project and she handles all correspondence and reports related to the project.

COOPERATORS

Numerous individuals have been contacted in order to ascertain whether they can collect mosquitoes for the project or can help with logistic matters related to the Principal Investigator's own field work. The following individuals have contributed to the project to date:

Maureen Coetzee and Richard Hunt, South African Institute for Medical Research, have provided information on collecting in South Africa and have promised logistic help and laboratory facilities when the Principal Investigator visits that country.

F. Glyn Davies, Veterinary Research Laboratory, Kabete, Kenya, has provided laboratory facilities, transportation, and field assistance during both collecting trips to Kenya.

S. D'Cruz and F.N. Mungaba, Department of Veterinary and Tsetse Control Services, Lusaka, Zambia, provided valuable help and transportation during the collecting trip to Zambia.

Thomas P. Gargan, II, United States Army Medical Research Unit - Kenya, obtained egg clutches of *Neomelaniconion* in May 1986, provided the Principal Investigator with advice and equipment for collecting soil samples, and processed the samples collected for *Aedes* eggs.

Bernard Geoffroy, ORSTOM, Paris, obtained egg clutches and specimens of *Neomelaniconion* in Ivory Coast during June and July 1986.

L.P. Lounibos, Florida Medical Entomology Laboratory, Vero Beach, Florida, obtained egg clutches of *Neomelaniconion* in Kenya during September 1986.

Michael W. Service, Liverpool School of Tropical Medicine, England, has provided valuable notes on *Neomelaniconion*

specimens in African and European museums and names and addresses of possible collaborators in Africa.

A.W. Sweeney, Army Malaria Research Unit, Ingleburn, Australia, sent specimens of _Neomelaniconion_ from Queensland.

Trevlyn Webb, Lilayi Farm, Lusaka, Zambia, provided valuable field help and transportation during the collecting trip to Zambia.

ACQUISITION OF SPECIMENS

Loans from Museums. - Major loans of _Neomelaniconion_ specimens have been received from the United States National Museum and the British Museum of Natural History. The latter loan is particularly valuable because it includes all the specimens studied and identified by F.W. Edwards during the preparation of his volume in the _Mosquitoes of the Ethiopian Region_ series (5).

Collecting and Rearing. - Mosquitoes were collected and reared in Kenya by T.J. Zavortink in June 1986 (42 collections), in Kenya by T.J. Zavortink and S.S. Shanks in November and December 1986 (72 collections), and in Zambia by T.J. Zavortink and S.S. Shanks in December 1986 (34 collections). Many specimens of _Neomelaniconion_ and associated species of ground-pool breeding mosquitoes have been obtained from these field surveys. However, attempts to collect topotypic material of _Neomelaniconion_ in the coastal strip of Kenya have not been successful to date.

During both collecting trips to Kenya, soil samples were dug with the hope that they contained viable eggs of species of _Neomelaniconion_. Single samples or multiple samples along a transect were taken from depressions that gave evidence that they held water during wetter seasons and from the edges of freshwater marshes and ponds that gave evidence of fluctuating water levels. Samples taken in June 1986 were placed into large plastic basins, flooded with tap water, and observed for larvae during the next 6-8 days. Only one of 27 samples yielded larvae of _Neomelaniconion_. Samples taken in November 1986 were subjected to the egg separation techniques used by T.P. Gargan, USAMRU - Kenya. None of 28 samples yielded _Neomelaniconion_ eggs. The Principal Investigator has concluded that taking soil samples is not an efficient way to obtain specimens of _Neomelaniconion_ for this project. It requires heavy or bulky equipment (shovel, mallet, template, mattock, basins) that is difficult to carry in the field; produces heavy bags of soil that are difficult to transport on public carriers, or basins of water that cannot be moved from locality to locality; and yields very few specimens.

A great number of progeny and individual rearings have been completed in the laboratory in San Francisco from egg clutches obtained in Africa. Included is material collected in Kenya during May 1986 by T.P. Gargan (58 clutches); in Kenya during June 1986 by T.J. Zavortink and F.G. Davies (11 clutches); in Kenya during September 1986 by L.P. Lounibos (6 clutches); in Kenya during December 1986 by T.J. Zavortink and S.S. Shanks (55 clutches); and in Ivory Coast during June and July 1986 by B. Geoffroy (17 collections). These progeny rearings have contributed extremely valuable specimens and data to the project. Through these rearings, the sexes and stages of each species reared have been unequivocally associated. Equally important is that these rearings have demonstrated that the species of Neomelaniconion are far more variable than previous workers have realized, and some females in these progeny series can not be identified accurately using characters utilized in existing published keys.

A standard procedure for treating all progeny rearings in the laboratory has been developed. Egg hatching and larval rearing are accomplished as follows: 1) eggs, still in the oviposition vial, are held in a humid chamber at room temperature for 24 hours, 2) the eggs are placed in a plastic cup and flooded with 100 ml of oxygenated distilled water at room temperature, 3) after one hour, 5 ml of 0.5% nutrient broth at room temperature are added to the plastic cup, 4) gaseous nitrogen is bubbled into the fluid in the plastic cup for 30 minutes, 5) the cup is placed into an incubator at 25°C, 6) after 24 hours, and for as long thereafter as necessary, small larvae are transferred from the cup with the eggs to a rearing pan, 7) the larvae are reared in plastic pans with aerated distilled water at 25°C and fed baker's yeast and desiccated liver powder as needed, 8) the cup with the eggs is held at 25°C until the water has evaporated and the eggs are dry, and 9) the eggs are subjected to the same standard treatment two more times.

Most eggs that hatch on a particular flooding hatch during the bubbling of nitrogen into the cup, or shortly thereafter, but some may not hatch until several days later. Most viable eggs of most clutches hatch during the first flooding, and only a few additional eggs hatch on the second and third floodings. However, with some clutches no viable eggs hatch during the first flooding, but many hatch on the second.

Larvae from most clutches grow rapidly and uniformly and produce vigorous adults. Larvae from some clutches, though, grow very slowly and irregularly, experience high mortality, and produce weak adults. Ascertaining the reason for this reduced survival is beyond the scope of the current project,

but the reason could be of biological significance. If the reduced survival is due to infection with transovarially transmitted arbovirus, then this fact is important to understanding the natural history of this virus. If the reduced survival is due to infection with some mosquito pathogen, then this pathogen may be valuable for biological control attempts.

PREPARATION OF SPECIMENS FOR STUDY

All material collected and reared by the staff in Kenya and Zambia during 1986 and the majority of the material reared in the laboratory in San Francisco from egg clutches have been prepared for study. Approximately 3500 adult mosquitoes have been mounted on points and approximately 2300 microscope slides of immature mosquitoes (whole larvae and larval and pupal skins associated with adults) have been prepared. Approximately 96% of these specimens (adults and slides) were provided with printed locality labels by the end of the contract year. Seventy microscope slides of male genitalia have been prepared.

IDENTIFICATION

All specimens of *Neomelaniconion* borrowed from the United States National Museum and the British Museum of Natural History have been sorted and identified as well as is possible with the existing literature. Nineteen species are represented in this material. These species and the geographic origins of the specimens are:

Aedes (Neomelaniconion)

- *albicosta* (Edwards) Ethiopia, Kenya, Somalia
- *albothorax* (Theobald) Kenya, Sudan, Tanzania, Uganda, Zaire
- *aurovenatus* Worth South Africa
- *bequaerti* Wolfs Zaire, Zambia
- *bolensis* Edwards Senegal
- *carteri* Edwards Nigeria
- *circumluteolus* (Theobald) Ethiopia, Ghana, Kenya, Malawi, Mozambique, Nigeria, Senegal, South Africa, Sudan, Uganda, Zaire, Zambia
- *crassiforceps* Edwards Zaire
- *fuscinervis* (Edwards) Gambia, Ghana, Liberia, Nigeria
- *jamoti* Hamon and Rickenbach Burkina Faso, Liberia
- *lineatopennis* (Ludlow) Australia, Burma, China, India, Indonesia, Malaysia, Philippines
- *luridus* McIntosh South Africa
- *luteolateralis* (Theobald) South Africa

mcintoshi Huang Angola, Ethiopia, Kenya, Nigeria, Zimbabwe, South Africa, Sudan, Tanzania, Zaire, Zambia
palpalis (Newstead) Central African Republic, Nigeria, Uganda, Zaire
pogonurus Edwards Zaire
punctocostalis (Theobald) Liberia, Nigeria
taeniarostris (Theobald) Ghana, Nigeria
unidentatus McIntosh Kenya, South Africa, Zimbabwe

During the first contract year, nine species of *Neomelaniconion* were collected and reared for the project. These species and their geographic origins are:

Aedes (Neomelaniconion)
circumluteolus (Theobald) Ivory Coast, Kenya
fuscinervis (Edwards) Ivory Coast
jamoti Hamon and Rickenbach Ivory Coast
mcintoshi Huang Kenya, Zambia
monotrichus Edwards Ivory Coast
punctocostalis (Theobald) Ivory Coast
taeniarostris (Theobald) Ivory Coast
unidentatus McIntosh Kenya
unidentified species Ivory Coast

Numerous other mosquitoes have been collected in Africa by the staff. These are primarily ground-pool breeding species collected in association with *Neomelaniconion*. Much of this material remains to be sorted and identified, but to date the following species have been determined:

Aedes (Aedimorphus)
cumminsii (Theobald) Kenya, Zambia
dalzieli (Theobald) Zambia
dentatus (Theobald) Kenya
eritreae Lewis Kenya
filicis Ingram and DeMeillon Zambia
fowleri (Charmoy) Zambia
hirsutus (Theobald) Zambia
ochraceus (Theobald) Kenya, Zambia
quasiunivittatus (Theobald) Kenya, Zambia
vittatus (Bigot) Kenya
Aedes (Albuginosus)
?*ngong* Van Someren Kenya
Aedes (Mucidus)
sudanensis (Theobald) Kenya, Zambia
Aedes (Skusea)
pembaensis Theobald Kenya
Aedes (Stegomyia)
aegypti (Linnaeus) Kenya, Zambia
bromeliae (Theobald) Kenya

calceatus Edwards Kenya
?deboeri Edwards Kenya
Culex (Culex)
antennatus (Becker) Kenya
decens Theobald Kenya
duttoni Theobald Kenya
pipiens Linnaeus Kenya
quinquefasciatus Say Kenya
simpsoni Theobald Kenya
Culex (Lutzia)
tigripes DeGrandpre and DeCharmoy Kenya
Eretmapodites
quinquevittatus Theobald Kenya
silvestris conchobius Edwards Kenya
subsimplicipes Edwards Kenya

ILLUSTRATION

Late in the contract year arrangements were made with Mary Ann Tenorio to start preparation of the illustrations for the monograph on _Neomelaniconion_. Ms. Tenorio nearly completed the preliminary drawings of the larva, pupa, and male genitalia of _Aedes (Neomelaniconion) jamoti_ by the end of the contract year.

The use of scanning electron microscopy for study and illustration of the eggs of _Neomelaniconion_ was explored. A suitable technique, based largely on that of Bosworth et al. (30), has been developed. Preparation of scanning electron micrographs of the eggs of all species of _Neomelaniconion_ for which eggs become available will be routine in the future.

TAXONOMIC STUDY

Acquisition of pertinent scientific literature is essential to taxonomic research, and so an exhaustive bibliography on _Neomelaniconion_ was started and copies of many of the most important papers and books that deal with _Neomelaniconion_ and other African mosquitoes were obtained for the project files.

Specimens of _Neomelaniconion_ borrowed from the United States National Museum and the British Museum of Natural History were identified with the existing taxonomic literature. These specimens, particularly those from the British Museum of Natural History, are extremely valuable because they help in understanding the species concepts of earlier mosquito taxonomists who have described _Neomelaniconion_, and because they determine how published names are to be applied to zoological species. However, most

of the specimens of Neomelaniconion in these major museums are not of suitable quality for modern taxonomic research. Many of the adults are in very poor condition, either shriveled, covered with fungus, dirty and/or greasy, denuded of scales and bristles, broken by careless handling, burst open by corroded insect pins, or nearly completely imbedded in adhesive. Very few of the adults have associated larval or pupal skins, and the skins that do exist are, for the most part, in very poor condition. Many males have had their genitalia removed and prepared for study, but the resulting preparations have been lost or are too poor to be useful. Many specimens, particularly in the United States National Museum, are without locality data. And, finally, a great many of the specimens are uniques and, therefore, provide no information on population variation. Because of the serious shortcomings of the existing collections of Neomelaniconion, it is obvious that the success of the "Biosystematics of Aedes (Neomelaniconion)" project depends to a great extent upon field work to acquire much new material of research quality.

Examination of Neomelaniconion borrowed from museums has shown the existence of two taxonomic problems that cannot be resolved with the existing specimens. One of these problems involves the status of populations of fuscinervis or a related species from Gambia and Liberia. Specimens from these countries are similar to typical fuscinervis in many ways, but have yellow scales on the margins of the scutum and at the base of some wing veins, and may represent an undescribed species. This cannot be determined, however, until long series of specimens with associated larval and pupal skins are collected in these countries and at the type locality of fuscinervis. The other problem involves the number of species related to, or similar in adult morphology to, palpalis, and the characters that may be of value in distinguishing these species. The nominal species involved in this complex are: palpalis, carteri, bequaerti, pogonurus, maculicosta Edwards, and punctocostalis. Again, long series of reared specimens with associated larval and pupal skins from the type locality of each of these taxa will be needed to resolve this problem.

Material collected and reared for the project "Biosystematics of Aedes (Neomelaniconion)" during the first contract year has been extremely valuable. All nine of the species are represented by adults with associated larval and pupal skins. Six of the species are represented by series of specimens in two or more collections, so that information on the existence and extent of intra- and interpopulation variation is available. Examination of the adults has shown that all six of these species are extremely variable, and

indicates that it will be very difficult to prepare a simple dichotomous key to identify females of Neomelaniconion. Study of the immatures has shown that while excellent diagnostic characters exist for some species, other groups of species have virtually identical larvae and pupae. In particular, no reliable characters for separating the immatures of circumluteolus, mcintoshi, and unidentatus have been discovered to date.

Examination of the eggs of Neomelaniconion at low magnification (up to 70X) with a stereoscopic microscope has shown that there are gross differences in shape between the eggs of some species. It is for this reason that scanning electron micrographs of the eggs of as many species as possible will be prepared. These micrographs will not only show the shapes of the eggs accurately, but also will allow study of the chorionic sculpturing. As seen with a stereoscopic microscope, the eggs of fuscinervis, jamoti, punctocostalis, and taeniarostris are narrowly fusiform, whereas those of circumluteolus, mcintoshi, and unidentatus are rhomboid.

Conclusions

The following are concluded as a result of the first year's activities of the project:

1. Collecting soil samples in an attempt to acquire eggs of Neomelaniconion for hatching and subsequent rearing of specimens is not an efficient field technique.
2. Collecting biting females in the field and rearing progeny in the laboratory from eggs they lay is the technique that yields the greatest number of reared specimens, the specimens of best quality, and the specimens of greatest taxonomic value.
3. Existing museum collections are so poor that it will be necessary to emphasize field work to try to collect research quality material of every species of Neomelaniconion specifically for the project.
4. The subgenus Neomelaniconion is very difficult taxonomically. Several species may occur sympatrically and each of these may be so variable that it may not be possible to prepare simple dichotomous keys for identifying females or larvae of every species.
5. Known taxonomic problems that must be resolved involve fuscinervis and palpalis and related and/or similar species.
6. Eggs of Neomelaniconion may provide valuable specific and/or group characteristics.

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